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Effect of pH and distillate volume on monitoring aroma quality of bittersweet chocolate

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Abstract

A central composite rotatable design (CCRD) was applied to study the response pattern for monitoring aroma quality of sweet chocolate bars. The two parameters varied were pH adjustment before distillation of chocolate and the distillate volume obtained after steam distillation. Aroma was qualitatively evaluated in three commercial chocolates and in their distilled extracts. Based on the responses for UV absorption, number of peaks on chromatograms and sensorial aroma values, response surface methodology (RSM) revealed that higher scores were obtained for each of these responses at a lower range (1.5–3) of pH and distilled volume (5–10 ml). It was also possible to distinguish between products of different manufacturers when a pH of 2.5 was adjusted for distillation and a distillate of 10 ml used. These conditions are considered optimum for monitoring the over-all aroma quality in cocoa products.

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Keywords: Chocolate; *Theobroma cacao*; Flavour analysis; Response surface methodology

1. Introduction

Cocoa (*Theobroma cacao* L.) is used as a raw material in many products and is highly appreciated for its flavour. Flavour formation in cocoa is related, partly to thermal treatment (roasting) during which several modifications occur, including the Maillard reaction, which is mainly responsible for the accentuated decrease in the concentration of reducing sugars and amino acids during roasting (Mermet, Cros, & Georges, 1992). In roasted cocoa, volatile compounds are dominated by alkyl substituted pyrazines followed by aldehydes and esters (Gill, MacLeod, & Moreau, 1984; Muggler-Chavan & Reymond, 1967; Oberparleiter & Ziegleder, 1997; Silwar, 1988; Ziegleder, 1991). Among the different classes of volatile compounds present in cocoa products, pyrazines are by far the most studied. Due to their pronounced formation during roasting, pyrazines are used as index compounds of the roasting process (Chaveron, Guyot, Hashim, Pezoa, & Pontillon, 1989; Hashim & Chaveron, 1994; Jinap, Wan Rosli, Russly & Nordin, 1998; Ziegleder, 1982; Ziegleder & Sandmeier, 1983).

Efficient techniques are necessary for investigating nitrogen heterocycles usually found at very low concentrations (Hérent & Collin, 1998). Pyrazines are extracted from cocoa beans and their products either by steam distillation followed by solvent extraction after pH correction, simultaneous distillation and extraction, direct solid/liquid extraction (Carlin, Lee, Hsieh, Hwang, Ho & Chang, 1986; Guyot, Gueule, Morcrette & Vincent, 1986; Mermet et al., 1992; Oberparleiter & Ziegleder, 1997; Reineccius, Keeney & Weisberger, 1972; Rizzi, 1967; Schnermann & Schieberle, 1997; Ziegleder & Stojacic, 1988). The two most important parameters, which influence aroma quality control in cocoa products, are pH adjustment before distillation and collection of a definite volume of extract after distillation. The pH adjustment procedure serves also to clean up the sample and to facilitate gas chromatographic analysis of cocoa volatile compounds (Reineccius et al., 1972). The determination of C_3–C_5 fatty acids was achieved with success by acidifying the cocoa sample before distillation (Lopez & Quesnel, 1973). In

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liquid/liquid extraction studies on cocoa, Reineccius et al. (1972) performed a clean up of the extract with pH 1 solution and then extracted the pyrazines at pH 8.3, while a direct extraction at pH 9 had also been reported (Mermet et al., 1992). However, in a model system, the best recoveries were obtained when liquid/liquid extractions were performed first at pH 0.1 and then at 12 (Hérent & Collin, 1998).

Ziegleder (1991) compared natural acid (pH 5.5–6.5) and alkaline (pH 8) cocoa extracts obtained by direct extraction. The acid extracted samples possessed a more intense and chocolate-like aroma than the alkaline ones. This behaviour was attributed to the high concentration of aromatic acids and sugar degradation products with their persistent sweet aromatic and caramel notes. Chocolate made from low pH (4.75–5.19) and high pH (5.50–5.80) cocoa beans presented low sensory response to chocolate flavour and more off-flavour descriptors were perceived from samples made from low-pH cocoa beans. On the other hand, chocolate samples made from medium pH (5.20–5.49) cocoa beans received a high response in strong chocolate flavour (Jinap, Dimick, & Hollender, 1995).

In the distillation methods followed by extraction, Ziegleder and Sandmeier (1983) reported that a 10 ml volume of distillate was sufficient for quantification of alkyl pyrazines and it had the benefits of just right volume avoiding any unnecessary dilution as well as having a reduced distillation period. This volume was also employed in other qualitative and quantitative studies (Oberparleiter & Ziegleder, 1997; Schnee & Eichner, 1987; Ziegleder & Stojacic, 1988). Other studies reported a distillate volume of 100 ml for phenolic compounds (Guyot et al., 1986), alkyl pyrazines (Chaveron et al., 1989; Mermet et al., 1992) and for pattern recognition of GC profiles of cocoa powder (Pino, Villavicencio, & Roncal, 1992).

Systematic studies related to extraction methods of cocoa volatiles are normally focused on alkyl pyrazines. Chaveron et al. (1989) performed comparative studies evaluating different methods and reported no difference in percentage recovery between steam distillation and simultaneous distillation and extraction (SDE). Ziegleder (1990) when comparing steam distillation, SDE and vacuum condensation found similar results in linalool content from flavour grade cocoas. A comparison between steam distillation and SDE for pattern recognition of GC profiles resulted in a higher extraction efficiency and an aroma closely resembling cocoa powder by steam distillation (Pino et al., 1992).

The UV activity at 278–280 nm (A_{280}) of the cocoa distillate is regarded as having the aroma-fullness and is used as an "aroma-index" or "flavour-index" for the roasting intensity (Serra BonvehI & Ventura Coil, 2000; Ziegleder & Sandmeier, 1983). Many compounds present in the distillate of cocoa nibs are responsible for contributing to A_{280}, among which 2-acetylfuran, 5-methylfurfural, 2-acetylpyrrol and alkylpyrazines possess pronounced effect (Ziegleder & Sandmeier, 1983). There exists a direct relationship between the A_{280} and pyrazine formation, especially trimethylpyrazine, during thermal treatment of cocoa nibs (Ziegleder & Sandmeier, 1983). In chocolate, the addition of vanillin and *p*-hydroxybenzaldehyde, also contribute to enhance the A_{280} (Ziegleder & Sandmeier, 1983).

The present work was therefore planned to study the effect of parameters such as pH of the sample and the distillate volume obtained on steam distillation on monitoring the aroma quality of bittersweet chocolate.

2. Materials and methods

2.1. Chocolate sample

Bittersweet chocolate bars of 500 g from three different producers were purchased from the local market at João Pessoa-PB, Brazil. The same batch of chocolate was used throughout the optimisation study. The bars were cooled, grounded, packed in 50-g portions and stored at −18 °C until analysis within a week.

2.2. Sample preparation

Thirty grams of ground chocolate were placed in a 500 ml round-bottom flask. Fifty grams of sodium chloride were dissolved in 200 ml of distilled water, added to the chocolate and mixed. The flask was heated to 60 °C for a complete sample homogenisation, followed by cooling to 25 °C. After cooling, the pH was adjusted with 1 M NaOH or concentrated HCl. Steam distillation was performed and the desired distillate volume (Vd) was maintained at 5 °C until solvent extraction. For extraction, 5 ml of distillate was saturated with NaCl and extracted 3 times, each with 3 ml of diethyl ether. The extracts were combined, dried over Na_2SO_4 and concentrated to 0.5 ml under a gentle stream of N_2.

After preliminary evaluation of the results, the final experimental conditions were fixed at a pH of 2.5 and a distillate volume of 10 ml.

2.3. Experimental design and statistical evaluation

A central composite rotatable design (CCRD) with two variables was used to study the response pattern and to establish a model (Barros Neto, Scarminio, & Bruns, 1996; Khuri & Cornell, 1996). The variables studied were pH and volume of distillate (Vd), each at five levels. Table 1 shows the symbols and levels. The dependent variables (responses) were UV absorption at 280 nm (A_{280}) and number of peaks on chromatograms detected.

Table 1
Experimental conditions for the variables (pH, distillate volume) and their coded levels for CCRD

Variables	Symbol	Levels				
		$-\alpha$	-1	0	$+1$	$+\alpha$
pH	X_1	1.0	2.5	6.0	9.5	11.0
Vd(ml)	X_2	5.0	10.1	22.5	34.9	40

The polynomial model used was:

$$Y = b_0 + b_1X_1 \ + b_2X_2 + b_3X_1^2 + b_4X_2^2 + b_5X_1X_2$$

where X_1 and X_2 represent the pH and volume of distillate (Vd), respectively, which were affected by the regression coefficients $(b_0, b_1, b_2, b_3, b_4$ and $b_5)$.

Results were analysed using response surface methodology (RSM). The selection of this particular design CCRD–RSM was based on the experimental objective to evaluate only a small number of selected variables. Among several statistical methods, RSM is designed to estimate interaction and even quadratic effects and therefore it gives an idea of the shape of the response surface being investigated. RSM designs are also most suited to find improved or optimal process settings and therefore this particular set of design was selected in this study.

To prepare adjusted models and their respective surfaces, the statistically non-significant parameters at $P \leqslant 0.05$, were excluded. The validity of the models was evaluated as a function of their respective coefficients of determination as well as by an analysis of the lack of fitness. Calculations were performed using the software package, Statistica version 5.0 (Statsoft, Tulsa, OK, USA).

For comparative evaluation of different samples, the results obtained were processed using analysis of variance of the software package, Statistica and the Tukey test applied for multiple comparisons at $P \leqslant 0.05$.

2.4. *UV analysis*

The "flavour-index" which is correlated to the cocoa aroma intensity could be monitored by measuring the UV activity at 278–280 nm (Ziegleder & Sandmeier, 1983). Many of the characteristic compounds formed during roasting and which belong to mainly furans and pyrazines are known to have their absorption at this wavelength. Thus the absorbency of the distillates was measured at 280 nm in a spectrophotometer (Beckman DU-600) The equipment was calibrated referring to a zero value for distilled water.

2.5. *Gas chromatography*

Extracts were analysed on a Hewlett-Packard model 5890 Series II gas chromatograph (Palo Alto, CA, USA) equipped with a flame ionisation detector. Gas chromatographic separation was performed on a fused silica capillary column, HP-INNOwax (30 m, 0.25 mm i.d. and 0.25 µl film thickness, Hewlett-Packard, Palo Alto, CA, USA). The carrier gas was helium at 1 ml min^1 flow-rate. Sample injection was carried out on a split mode (1:25). Volumes of 5 µl of concentrated extracts were injected. The injector and the detector temperatures were 220 and 250 °C, respectively. The oven temperature was initiated at 30 °C for 5 min, raised at a rate of 7 °C/min to 100 °C, then at 1 °C/min to 130 °C, and finally at 10 °C/min to 195 °C wherein maintained for 18 min.

2.6. *Sensory evaluation*

Chocolate aroma was evaluated in all commercial products and in their extracts. The panel consisted of nine selected and trained members. The panel members completed five training sessions prior to data collection. In the training sessions the panel members received a reference sample for "strong" (Nestlé Classic bittersweet chocolate, 2 g in amber vials, Nestlé Brasil Ltda, Caçapava-SP., Brazil) and other samples of deodorised cocoa butter for "none". The training sessions enabled panel members to become familiar with the scale and sensory properties of the aroma. It also helped to reduce variability between and within panel members ($P < 0.05$). In all tests, the members marked on a 10 cm scale related to the intensity of chocolate aroma. The scale was anchored at left with "none" and at right with "strong". The difference between chocolate brands was evaluated by Multisample Difference Test, rating approach, and by analysis of variance (Meilgaard, Civille, & Carr, 1987). This test was applied to determine in which way a particular sensory attribute varies over a number of *t* samples, where *t* may vary from 3 to 6 or at most 8, and it is possible to compare all *t* samples as one large set (Meilgaard et al., 1987). Each extract containing 10 µl was spiked on a strip of filter paper and after solvent evaporation (approximately 20 s), placed inside a 50-ml amber vial. Vials were left for 15 min at ambient temperature (25 °C) for aroma equilibration and then presented to the panel members. Results were evaluated by RSM as described earlier.

3. Results and discussion

Experimental conditions for different extracts obtained and their responses for absorbance, number of peaks on chromatographic analysis and sensorial aroma notes are shown in Table 2. The evaluation of the effects on absorbance indicated that all linear and quadratic terms were significant ($P < 0.05$; Table 3). There was no significant interaction between pH and Vd. The negative

Table 2
Experimental design and the responses for absorbance, number of peaks and chocolate aroma of the extracts

Experiment	Coded values		Responses		
	X_1 (pH)	X_2 (Vd)	Absorbance (280 nm)	Number of peaks	Aroma[a]
1	-1	-1	1.812	94	7.7
2	$+1$	-1	1.367	22	4.0
3	-1	$+1$	1.103	24	5.2
4	$+1$	$+1$	0.759	76	3.6
5	$-\alpha$	0	1.547	105	6.0
6	$+\alpha$	0	0.966	80	4.2
7	0	$-\alpha$	1.533	105	6.9
8	0	$+\alpha$	0.797	93	3.2
9	0	0	0.965	36	5.9
10	0	0	1.021	34	5.5
11	0	0	0.958	17	5.4

[a] Rating values on a 10 cm scale, 0 = none and 10 = strong chocolate aroma.

Table 3
Estimate of the effects and regression coefficients for the absorbance, number of peaks and chocolate aroma, for the codified variables

Factor	Effect	Coefficient	Standard error	*P*
Absorbance				
Mean interaction	0.981	0.981	0.086	0.0004
pH (*L*)[a]	−0.403	−0.201	0.105	0.0037
pH (*Q*)[b]	0.300	0.150	0.125	0.0093
Vd (*L*)	−0.589	−0.295	0.105	0.0017
Vd (*Q*)	0.208	0.104	0.125	0.0189
pH × Vd	0.051	0.025	0.048	0.2811
Number of peaks				
Mean interaction	29.00	29.00	2.20	0.0406
pH (*L*)[a]	−13.84	−6.92	21.56	0.2017
pH (*Q*)[b]	42.63	21.31	25.65	0.0399
Vd (*L*)	−8.24	−4.12	21.55	0.3803
Vd (*Q*)	49.13	24.56	25.66	0.0305
pH × Vd	62.00	31.00	30.49	0.0272
Aroma				
Mean interaction	5.60	5.60	0.66	0.0007
pH (*L*)[a]	−2.31	−1.16	0.80	0.0065
pH (*Q*)[b]	−0.65	−0.33	0.35	0.1000
Vd (*L*)	−2.38	−1.19	0.81	0.0061
Vd (*Q*)	−0.70	−0.35	0.45	0.0880
pH × Vd	0.35	0.18	1.13	0.3168

[a] *L*, linear coefficient.

[b] *Q*, quadratic coefficient.

linear effect of both pH and Vd indicated that an increase in these values contribute to a lower absorbance. It has been reported that increasing the distillate volume results in a decrease in pyrazine concentration (Ziegleder & Sandmeier, 1983). For the response number of peaks, the linear parameters were not significant ($P > 0.05$), while the quadratic and interaction terms were significant (Table 3). The variable chocolate aroma was significantly affected only by the linear terms (Table 3). As for the absorbance, the variable chocolate aroma was influenced negatively by pH and Vd while for the chocolate aroma score, a pH change from 9.5 to 2.5 at fixed Vd of 10.1 ml led to an increase in aroma score by 3.7 (Table 2). The same was observed for Vd, where a change from 34.9 to 10.1 ml at a fixed pH of 2.5 led to an increase in chocolate aroma score by 2.5 (Table 2). Ziegleder (1991) reported that steam distillates of cocoa are characterised by sharp odours, while acid extracts obtained by direct extraction revealed pleasant and persistent cocoa and chocolate-like notes. It is clear from these results that pH also influenced the aroma perception of extracts obtained by steam distillation followed by extraction.

The usefulness of the CCRD experimental design for the study of the distillation process is evidenced by the finding of significant interaction between pH and Vd, and curvature effects (quadratic terms) which means that an approach considering one-factor-at-a-time would fail due to the presence of the second order terms. However, this experimental design allowed us to obtain a better representation of effects and interactions that could be easily interpreted by three-dimensional graphics representing response surfaces. The RSM allows the estimation of the tendencies to highest and/or lowest values of the dependent variables (Box, Hunter, & Hunter, 1978; Cochran & Cox, 1957).

Furthermore, the differences observed in the number of peaks obtained on chromatographic analysis of extracts were not only due to a quantitative effect, but also mainly qualitative, since several chromatogram profiles were obtained. Buttery and Ling (1995) studying volatile flavour components of corn tortillas by dynamic headspace conditioned the sample at pH 4.5–6.4, 7.4 and 9.4–9.8 reported a better recovery from the bases under the mild alkaline (pH 7.4) conditions. The main differences were not found in the profile, but in the recovery factors. For instance, pH effect in headspace analysis is not so obvious, since other factors such as the sampling time and salt concentration could influence the results of pyrazine extraction (Baudron, Belin, & Voilley, 1991).

The parameters found to be significant at a probability level (*P*) equal or less than 5% were considered to obtain the fitted models to predict the responses. Table 4 shows that the models for absorbance, number of peaks and aroma presented coefficients of determination of 0.98, 0.67 and 0.85, respectively, indicating that nearly 98, 67 and 85% of the total variation can be explained or accounted for by the models. The coefficient of determination for the absorbance was higher than that for the sensorial aroma notes and number of peaks, although the latter is also satisfactory, considering the values normally used in gas chromatographic analysis. All models presented a nonsignificant lack of fitness ($P > 0.05$).

Table 4
Refined response surface equations with regression coefficients for absorbance, number of peaks and chocolate aroma

Responses	Model	R^2	P (lack of fit)
Absorbance	$Y = 2.640 - 0.201\ \text{pH} - 0.055\ \text{Vd}^{a} + 0.012\ \text{pH}^2 + 0.001\ \text{Vd}^2$	0.98	0.226
Number of peaks	$Y = 68.29 - 0.22\ \text{pH}^2 - 0.01\ \text{Vd}^2 + 0.08\ \text{pH Vd}$	0.67	0.125
Aroma	$Y = 9.24 - 0.33\ \text{pH} - 0.09\ \text{Vd}$	0.85	0.109

[a] Vd, distillate volume (ml).

In accordance with the fitted models, a response surface was constructed for the distillates for the absorbance, the number of peaks and sensorial aroma notes (Figs. 1, 2 and 3, respectively). Fig. 1 shows that working in a combination of low pH and low distillate volumes, a higher value for the absorbance was obtained. It also can be depicted from the curvature that the increase in absorbance due to pH and Vd reduction was intense. The response surface obtained for the number of peaks (Fig. 2) also indicated that a combination of a lower range of pH and Vd produced a higher number of peaks. In this case the increase was slower and tend to stabilise, which can be observed by the downward concavity of the surface. The highest scores for sensorial aroma were obtained at a lower range of pH and Vd (Fig. 3). The surface was a plane since only the linear terms were significant, but as for the other dependent variables there was an increase in the response at the lower range of pH and Vd. It is of interest to note that despite the different shape of the surface (different functions) the maximum of absorbance, number of peaks and aroma score were coincident at the low levels of pH and Vd. A comparative analysis of the three figures reveals the tendency of the responses being at low pH and lower distillate volumes and it has an advantage of saving time in aroma quality evaluation. Another important point in surface analysis is that as aroma is most intense at low pH and lower Vd: the chromatographic peaks obtained in the same region could be of major aroma impact compounds. Furthermore, we observe also that the region of more absorbency coincides with the region of intense aroma and a greater number of peaks. Ziegleder (1991) reported that acid extracts obtained by direct extraction revealed pleasant and persistent cocoa and chocolate-like notes. It is clear from these results that pH also influenced the

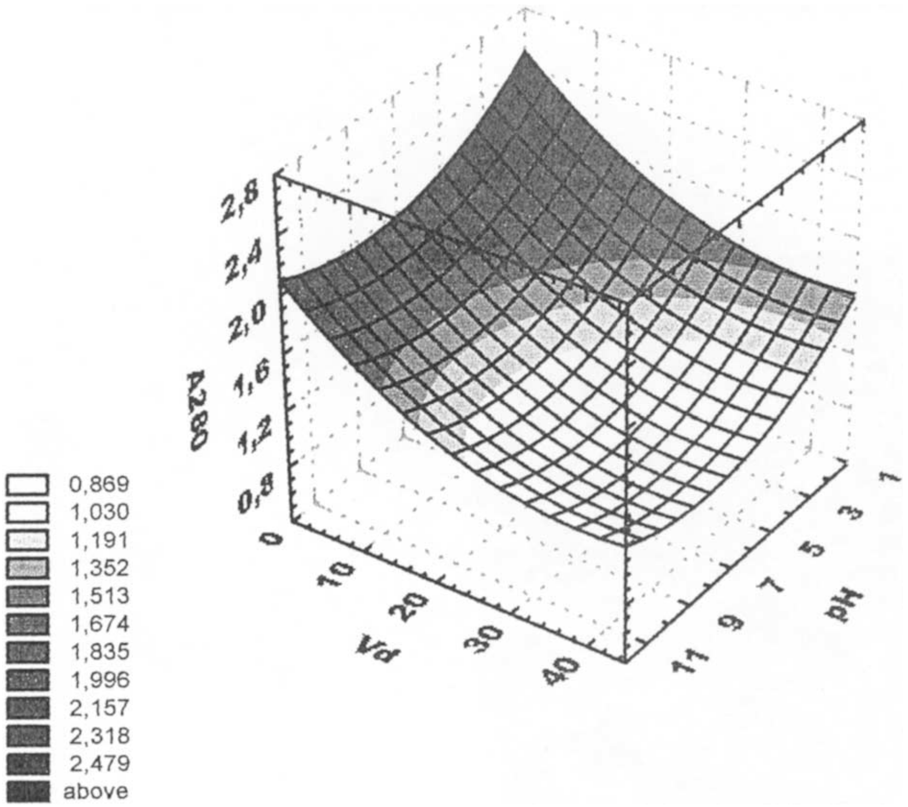

Fig. 1. Response surface for the absorbance of the distillate as a function of pH and the distillate volume.

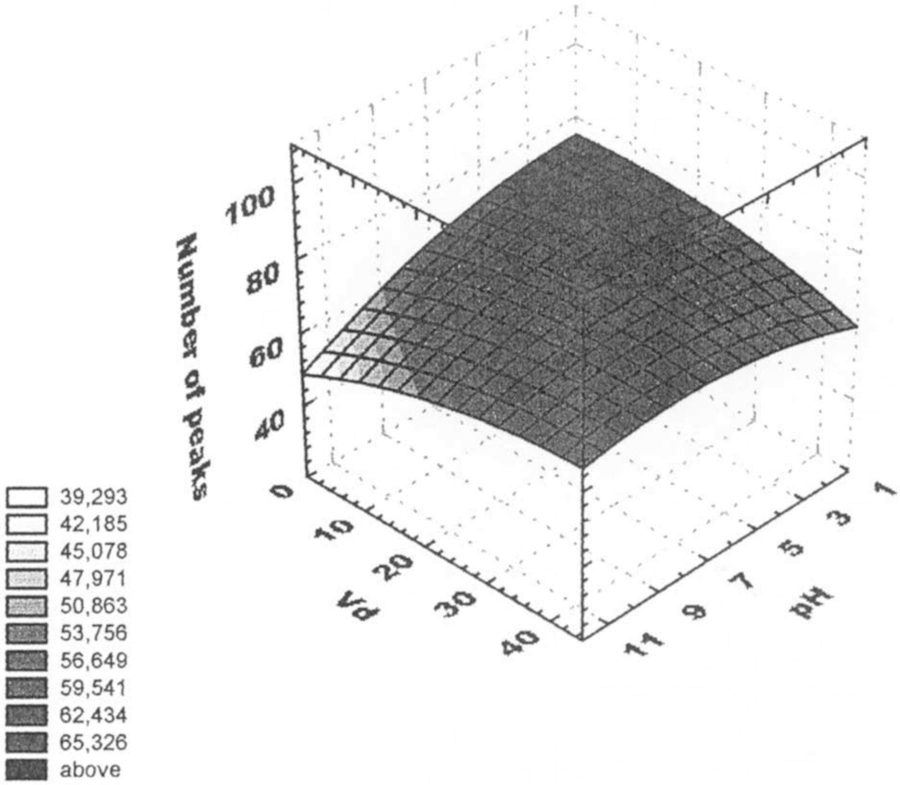

Fig. 2. Response surface for the number of peaks as a function of pH and the distillate volume.

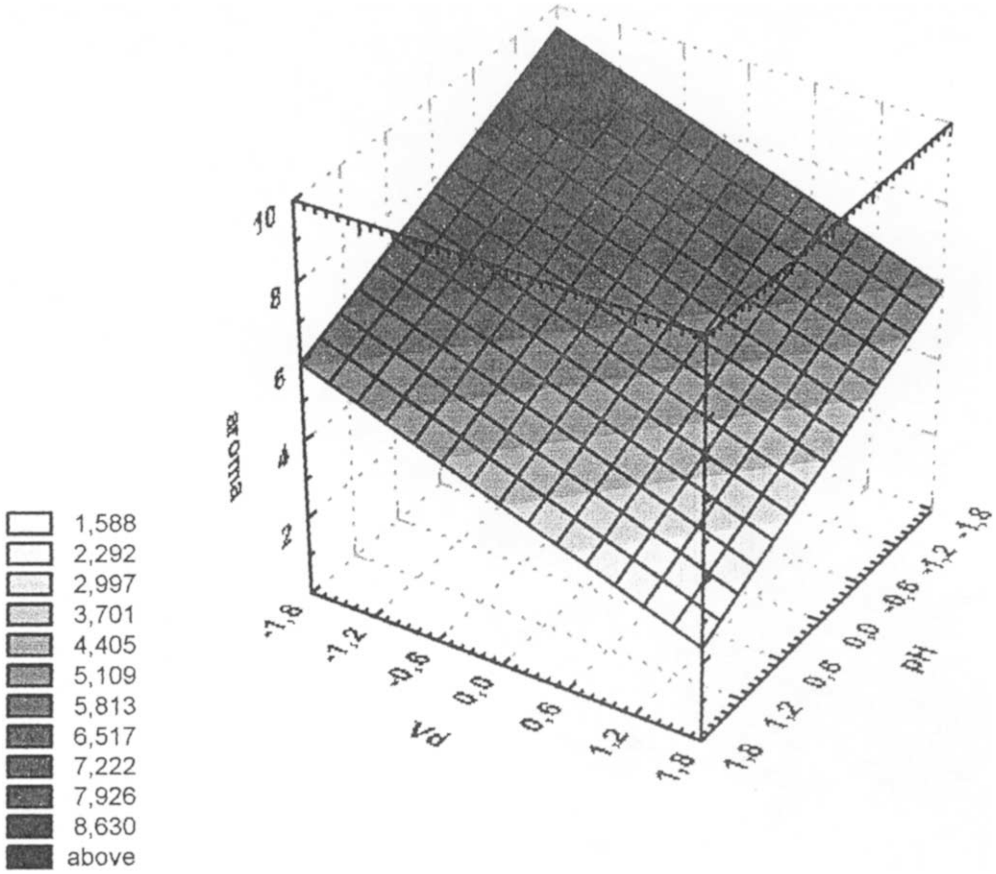

Fig. 3. Response surface for the chocolate aroma as a function of pH and the distillate volume.

Table 5
Values for absorbance (mean ± standard deviation), number of peaks and chocolate aroma obtained from different Brazilian bitter sweet chocolates (samples A, B and C) and of their extracts on distillation[a]

Samples	A_{280}	Number of peaks	Aroma from chocolate	Aroma from extract
A	1.271a ± 0.004	100	9.3a	6.3 a
B	0.961b ± 0.001	81	6.6a	5.0 a,b
C	0.795c ± 0.002	28	1.5b	4.0 b

[a] Means with different letters within a row are significantly different ($P \leqslant 0.05$).

aroma perception of extracts obtained by steam distillation followed by extraction.

In the study on different bittersweet chocolate brands, we decided to perform distillation with low distillate volume (10 ml) and at a lower pH (2.5). Results for absorbance, number of peaks obtained on gas chromatographic analysis and sensorial notes for aroma evaluation of different products and their extracts are presented in Table 5. The three samples analysed presented significant differences for the absorbance indicating, as also observed by Kleinert (1983), that we could distinguish products of different producers. Two samples presented an intense chocolate aroma while one sample possessed a weaker aroma, which also had the lowest absorbance. Chocolate aroma scores of the flavour extracts, despite the lower intensity were in good agreement with the products scores. Moreover, for the number of peaks, there was also a marked difference between samples.

Although our results were obtained with bittersweet chocolate, we recommend that care should be taken using absorbance (at 280 nm) to compare quality index of cocoa beans and cocoa products from different origins, since cocoa pH could vary from 4.65 to 5.97 (Jinap et al., 1995) and cocoa powder from 5.22 to 6.84 (Serra Bonvehí, & Ventura Coll, 1997) and this variable (pH) significantly affects the UV activity of the distillate.

Using the method of experimental CCRD and response surface analysis it was possible to determine optimal operating conditions to obtain good quality aroma extracts. In the conditions used in these experiments, pH range varying from 1.5 to 3 and distillate volume from 5 to 10 ml presented the best performance for over-all aroma quality. Based on these results, we strongly recommend that pH should be controlled in cocoa flavour extraction by distillation for monitoring the over-all aroma quality in cocoa products.

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